WEB WATCH

Networking with proteins

Creating user-friendly databases of protein–protein interactions is a tough challenge. Pronet — a curated database of published protein–protein interactions produced by Myriad Genetics — is a brave attempt to address the problem.

Searching the database couldn't be simpler: you just type in a keyword and hit 'go'. This returns a list of proteins whose database entries contain that word. Clicking on any entry in this list will take you to that protein's homepage, which contains a graphical representation of the protein's domain structure, links to other relevant databases, and sequence information. But Pronet's killer application is the addictive 'view interactions graphically' feature, which takes you to a page containing your protein in a box. Clicking on the box creates a spider diagram showing all the proteins it interacts with, and you can expand the network as far as you like. A 'mouse mode' menu allows you to delete proteins, move them, or squeeze the network to make room for more interactions. What's more, if you come across a protein you're unfamiliar with, choosing 'info' from the mouse mode menu will link you back to that protein's data entry page.

Pronet does have limitations: it contains only human sequences, although there are plans to include other species. It also records only interactions found using the yeast two-hybrid system, which creates some idiosyncrasies. For example, the c-Src page states that there are 'no recorded interactions for this protein'. Links to papers describing the interactions would also be useful. That said, as more data are added, Pronet will evolve into an invaluable tool for anyone wanting to track protein–protein interactions.

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▶cells that aren't cycling. Similar results were obtained in the salivary gland, an organ that undegoes endoreduplication (cell division without cytokinesis).

Datar and colleagues turned back to the wing to study whether CycD–Cdk4 exerts its effects through Rbf. As expected, overexpression of Rbf alone slowed cell division, whereas cells expressing all three proteins had near normal cell divison rates but were larger, indicating that cell growth was promoted even while the cell cycle was being slowed by Rbf. In the eye, by contrast, Rbf overexpression didn't influence postmitotic growth, and insertion of a null *Rbf* allele had no effect on cell growth in either the wing or the eye. CycD–Cdk4 must, therefore, be promoting cell growth by phosphorylating targets other than Rbf.

So, rather than being dedicated to getting cells through G1, CycD–Cdk4 promotes hyperplasia (increased numbers of cell divisions) in dividing cells, hypertrophy (increased cell size) in endoreduplicating cells and both in postmitotic cells — and it doesn't need Rbf to carry out any of these functions. The solution to the next challenge — determining the growth-promoting target of CycD–Cdk4 — might well have all our eyes bulging.

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References and links

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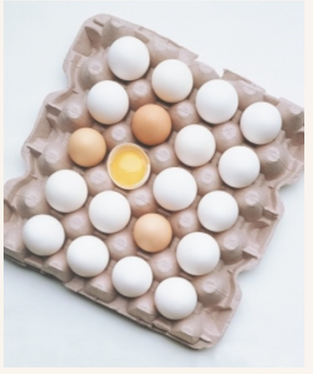
FURTHER INFORMATION Cyclins and E2F: a Kohn interaction map | The interactive fly: cell cycle genes | Mitosis world

PROTEOMICS

This way up and handle with care

Proteins are beastly to work with: they denature at the drop of a hat and have an annoying tendency to regulate their social interactions by post-translational modifications. Small wonder, then, that researchers wanting a high-throughput readout of cellular behaviour use DNA microarrays to look at messenger RNA levels instead, even though they don't necessarily correlate with protein activity. All that might be about to change: in the 8 September issue of *Science*, Gavin MacBeath and Stuart Schreiber report that they can make microarrays of functionally active proteins, and can use them to measure interactions with other proteins and small molecules.

Two hurdles had to be leapt: keeping the proteins active and getting them in the right orientation. A third goal was to make the technology compatible with existing microarray analysis tools. With hindsight, the solutions to these problems turned out to be laughably simple: use the gear that prints commercially available DNA microarrays, put 40% glycerol in your buffers to prevent dehydration of the nanolitre volumes applied, and coat your slides with a reagent that reacts with primary amines. This



captures proteins by their amino termini or by surface-exposed lysine residues, so each protein gets stuck to the glass in a range of different orientations, one of which is almost bound to be the right way up. The slides are then quenched with bovine serum albumin (BSA) which, as well as blocking any unreacted groups, lowers background noise when the slides are probed with other proteins. These simple tricks have allowed MacBeath and Schreiber to print proteins at densities of 1,600 spots per square centimetre.

Now pick your favourite protein function. Do you want to find new protein–protein interactions? Or hunt for new substrates for your pet protein kinase? Or are you more interested in finding out what proteins your library of drug candidates binds to? The researchers did proof-of-principle experiments to show that all of these applications are feasible by flooding the slides with fluorophore-tagged proteins, kinase substrates in the presence of radiolabelled ATP, or synthetic ligands coupled to fluorescently labelled BSA. Although most of these experiments were done using a small number of arrayed protein spots, they also work in the context of a chip containing over 10,000 spots: a single spot of the FKBP12–rapamycin binding protein (FRB) can easily be located in a sea of protein-G spots when probed with a mixture of two fluorophore-tagged proteins — one binding to FRB, the other binding to protein G.

The greatest barrier to commercial availability of these protein microarrays will be purification of the proteins to put on them. Let's hope that the current trend in automation obviates the need for arrays of protein biochemists, cursing in cold rooms over jammed fraction collectors.

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